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(54) Purifying proteins by adsorption thereof on a carrier

(57) A process for purifying proteins especially enzymes which are directed to substrates which contain aromatic groups, in an aqueous medium, by adsorption, the proteins being fixed to a specific carrier material by hydrophobic adsorption, this carrier material containing phenylbutylamine groups covalently bound to the carrier material is described. Penicillinamidase may be immobilised by adsorption on the carrier, followed by treatment with a cross-linking agent.

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PROCESS FOR PURIFYING PROTEINS

The invention relates to a process for purifying protein, particularly enzymes from biomass, especially the enzyme penicillinamidase.

It is known to purify proteins, particularly enzymes, by hydrophobic chromatography. Thus, for example, the enzyme penicillinamidase (penicillinacylase PcA) from E.coli (E.C. 3.5.1.11), which is used for the industrial conversion of penicillins produced by fermentation in 6-aminopenicillinic acid (cf. H. J. Rehm & G. Reed in Biotechnology, vol. 7a, pg. 168, 169 VCH 1987), can be purified on phenylglycinsepharose by affinity chromatography (Indian Patent No. 155051; Purification of Penicillin Acylase Enzyme; P. S. Borkar and P. B. Majahan).

Furthermore, benzyl-EUPERGIT is used for the purification of PcA by affinity chromatography. Affinity chromatography using affinity ligands consisting of phenylacetic acid or derivatives thereof is described in Chem. Abstr. 97, 353 63a.

Other studies have used conjugates of agarose or sepharose with ampicillin or amoxycillin as matrices for affinity chromatography (Indian Patent No. 155050; P. B. Mahajan et al. Hind. Antibiot, Bull. 24 (1-2) 38, 1982, Chem. Abstr. 97, 87551e and Chem. Abstr. 95, 110 740 K).

However, the processes which have hitherto been proposed are without exception expensive, both in terms of the number of individual operations needed and also in terms of the apparatus required. The increased amount of time involved is a disadvantage for the natural qualities of

the protein which is to be purified.

We have now developed a process for purifying or isolating protein which enables purification to be achieved as fast as possible, with substantially no waste and under gentle conditions.

Thus, according to one aspect of the present invention, there is provided a process for purifying proteins which are capable of binding to substrates which comprise aromatic group, said process comprising fixing said proteins from an aqueous solution thereof by hydrophobic adsorption onto a specific carrier material having phenyl butylamine groups covalently bound thereto.

According to a further aspect of the present invention, there is provided a carrier material for fixing proteins, said carrier material having phenyl butylamine groups covalently bound thereto as the product of the reaction of a glycidyl group as the activated group of a carrier precursor T-Vs-A.

The invention will be more particularly described with reference to a process for purifying enzymes which are directed to substrates which contain aromatic groups, particularly phenyl groups. More preferably, the invention relates to a process for purifying the enzyme penicillinamidase (PcA) (cf. E.C.3.5.1.11), particularly the enzyme from E.coli or from B.megaterium. In the process according to the invention the protein which is to be purified is fixed by hydrophobic adsorption on a specific carrier T, containing phenylbutylamine (which has been covalently bonded to the carrier by reaction with an active group of the carrier material).

The carrier material may be of natural or synthetic origin, and the active groups of the carrier material

should conveniently react with phenylbutylamine via an electroneutral bonding mechanism. Consequently, there should be no change in the electrochemical nature of the ligand phenylbutylamine.

This requirement is satisfied particularly favourably, for example, by a copolymer consisting of the matrix component (meth)acrylamide, a crosslinker, e.g. N,N-methylene-bis-(meth)acrylamide and the active binding components glycidyl(meth)acrylate and/or allyl-glycidylether. Corresponding products are commercially available under the trade mark ® EUPERGIT C (made by Röhm GmbH). Other carrier materials are also suitable, e.g. crosslinked agarose substituted with epoxy groups ("epoxy activated ® SEPHAROSE") and synthetic polymer carriers based on crosslinked polyvinylacetate, the acetyl groups of which have been hydrolytically removed and substituted with epoxy groups. (VA-epoxy carriers made by Riedel-De Haen). Suitable carriers (T-PbA) are also obtained if the above-mentioned carrier materials are substituted with tresyl groups (K. Nilsson and K. Mosbach, Biochem. Biophys. Res. Communications, vol. 102, No. 1 pp. 449-457 (1981)) instead of the epoxy groups as the active groups (for example "tresyl activated ® SEPHAROSE" or tresyl activated ® EUPERGIT-diol).

The carrier material (T-PbA) which can be used for protein purification may be prepared in a manner known per se: (the carrier material precursors are hereinafter designated T-VS, and the activated form is referred to as T-VS-A). The active binding component (active group) may be introduced either by polymer-analogous reaction (e.g. by the addition of epichlorohydrin to hydroxyl groups of the polymer) or by direct copolymerization of monomers containing these active binding groups.

The most suitable matrix monomers are electroneutral, hydrophilic vinyl compounds such as (meth)acrylamide, whilst the amide group may optionally be substituted with (hydroxyl-substituted) C_1-$_4$-alkyl groups, as well as hydroxyalkyl esters with C_{1-6} alkyl groups in the ester group of the (meth)acrylic acid, e.g. esters of polyols, and also vinyl alcohols, allyl alcohol, vinyl esters such as vinyl acetate or vinyl propionate. The proportion of monomers in the carrier material which contain the active binding component is generally between 4 and 40% by weight. The proportion of crosslinking monomer component is generally between 5 and 80% by weight. The proportion of the matrix monomer makes the total up to 100% by weight.

Suitable crosslinking monomers are the crosslinkers known per se, preferably electroneutral, hydrophilically crosslinking monomers such as N,N'-methylene-bis-(meth)acrylamide, polyols which are polyesterified, but at least diesterified, with (meth)acrylic acid, particularly pentaerythritol (meth)acrylic acid esters of this type.

The density of ligands is expediently 10-2000 µMol PbA groups per gramme of carrier material in the dry state, preferably 50-500 µMol.

In the context of the present invention, there is particular interest in carrier materials having a macroporous structure and preferably a spherical configuration, to which the phenylbutylamine group is covalently bonded (cf. Jahrbuch der Biotechnology, 1986-1987, S. 404, Carl-Hanser-Verlag). Macroporous structures of this kind are found for example in the commercial products of the ® EUPERGIT C type (EUPERGIT C, Eupergit C3ON, EUPERGIT C250 L), as well as in ® FRACTOGEL or ® TOIOPEARL and in corresponding

macroporous ion exchangers (e.g. ® LEWATITE made by Bayer AG, and AMBERLITE made by Röhm & Haas) after suitable conversion into electroneutral material.

As already mentioned, the (activated) carrier material precursors T-VS-A of the same kind as ® EUPERGIT C. are of particular interest. This material is a crosslinked copolymer of acrylamide or methacrylamide and glycidyl acrylate or methacrylate, preferably consisting of bead-shaped particles. Matrix polymers of this kind are described in DE-C-2722751 or US-A-4190713 and US-A 4 511 694. The beads generally have a diameter from 5-1000 μm, more particularly 30-1000 μm. A value of 0.8-2.5 μMol per mg of dry carrier material may be mentioned as a guide to the content of glycidyl groups in the carrier material T-VS-A which can be reacted. Other characteristics will become apparent from the following table.

Characteristics	Data
Average particle size:	140-180 μm
Pore diameter:	40 nm
Exclusion limit = M_{LIM}:	2.10^5 Daltons
Active binding surface:	180 m^2/g (dry)
Epoxide content:	800-1000 mmol/g (dry)
Water uptake	2.3 ml/g (dry)
Density = d_4^{20}:	1.20
Bulk density:	0.34 g/ml

Characteristics	Data
Binding capacity: (under conventional conditions)	
Human albumin:	48 mg/g (wet)
Human IgG:	34 mg/g (wet)
The swelling characteristics in the presence of water:	1 ml (dry) gives 1.4 ml (wet)
Solubility (in water, buffers and organic solvents)	insoluble
Pressure stability:	300 bar

Under the electron microscope it is possible to see the macroporous structure of the beads with channels and cavities having a diameter of from 0.1-2.5 μm (1000-25000 A), so that enzyme or substrate molecules ranging in size from 10-100 Å can reach everywhere inside the macroporous matrix.

The ligand responsible for purifying the protein is conveniently prepared by reacting the carrier material precursor T-VS-A with the amine phenyl butylamine. Generally, the reaction may be carried out by directly reacting the carrier material which contains epoxy groups, for example, with phenyl butylamine, preferably in a suitable liquid medium, e.g. water or an alcohol such as ethanol. Conveniently, the amine is used in a certain excess, based on the functional groups of the T-

VS-A. The reaction is preferably carried out at elevated temperature, e.g. in the range from 30-100°C. After the reaction the excess amine is removed by treatment with acid, for example dilute sulphuric acid, and washing with water. More specifically, the bonding of the phenyl butylamine to the carrier material precursor T-VS-A may be carried out as follows. In a suitable inert liquid medium such as ethanol or a ketone such as acetone, the carrier material precursor T-VS-A is mixed with preferably an excess (based on the number of active binding groups in the precursor material) of phenyl butylamine, e.g. up to a 5-fold excess but preferably about a 2-fold excess, and the mixture is reacted for a certain length of time, depending on the temperature selected, which may be between room temperature and about 100°C, for example at the boiling temperature of the medium, for generally between 3 and 72 hrs. The product is then washed with the solvent and then with water in order to remove any excess amine, the organic medium making up 5-15 times the weight of the dry carrier material, whilst the water constitutes 10-30 times the weight of the dry carrier material. Then washing is repeated with a dilute inorganic acid, especially 0.5 M sulphuric acid (3-5 times the weight of the dry carrier material) and the mixture is optionally refluxed for 10-30 minutes in 0.5 M sulphuric acid (3 times the quantity by weight), then washed with water until a neutral reaction is achieved.

The reaction of the carrier material T-PbA with the protein, especially when an enzyme, more particularly an enzyme of the penicillinamidase type, may be carried out generally as follows: The homogenate from the enzyme production is preferably sedimented in a cooling centrifuge, e.g. at 5000-10000 g, then the (clear) supernatant, which usually contains 15-20 units of penicillinamidase per ml, is poured into a

chromatography column charged with the carrier material T-PbA and filled with a buffer solution of buffer A (for example 0.05 molar potassium phosphate buffer pH 7.5 plus 1 mol NaCl). A column charged in this way can be charged with 30-100 units of penicillinamidase per ml of moist carrier, resulting in 10-80 mg of protein per ml of carrier. The columns have a diameter ranging from 1 mm to 30 cm and a height from about 10 cm to 1 m or more. It is advisable to keep within a flow rate (linear flux) of 60 to 100 cm per hr. The column is rinsed with buffer A and the enzyme is then eluted with an elution buffer (0.1 mol of sodium formate buffer, pH 3.8, in the case of penicillinamidase) and then taken up in 0.1 volume of a 1 molar potassium phosphate buffer, pH 7.5. Generally, the elution process is complete in 5 to 60 minutes, preferably about 20 ± 10 min, depending on the column. The eluate is suitable, for example, for use directly in immobilization, particularly covalent immobilization on a solid carrier, e.g. EUPERGIT C. The immobilization yield is about 80% and the product activity is 100 units per gramme of moist weight.

The same column can be used at least 50 times in succession for purifying the protein/enzyme, provided that, after each running, the column is treated with 2 to 5 volumes of the column of a protease solution, preferably with 0.006 Anson units, at pH 10. Suitable proteases are preferably alkaline proteases, particularly those of bacterial origin. (Cf. Keay, Process Biochemistry, 17-21, 1971; H. J. Rehm, G. Reed Biotechnology vol. 7a, loc.cit pg. 156-168; Ullmann's Encylopädie der Techn. Chemie, 4th edition, vol. 10,517-522, Verlag Chemie 1975).

Particular mention may be made for example of alkaline proteases obtained from Bacillus subtilis and also those obtained from Aspergillus sp. particularly A. oryzae.

The proteolytic activity of the enzymes may expediently be determined by the so-called Löhlein-Volhard method ("the Löhlein-Volhard method of determining proteolytic activity", Gerbereitechnisches Taschebuch, Dresden-Leipzig 1955) and is given or determined in "LVU" (Löhlein-Volhard units). An LVU unit is the quantity of enzyme which digests 1.725 mg of casein under the specific conditions of the method. For determining the activity of enzymes which are effective in the acid range, which is derived from the Anson method (M. L. Anson, J. Gen. Physiol 22, 79 (1939)), the following applies: the units are referred to as "protease units (hemoglobin)" (= U_{Hb}). A U_{Hb} corresponds to the quantity of enzymes which catalyses the release of trichloroacetic acid-soluble fragments of hemoglobin equivalent to 1 Mol of tyrosin per minute at 37°C (measured at 280 nm). 1 mU_{Hb} equals $10^{-3}U_{Hb}$.

The advantageous effects compared with the prior art consist, inter alia, in the exceptionally high binding capacity of the carrier material according to the invention. This also results in the possibility of immobilizing the protein directly on the carrier by crosslinking, thereby resulting in a product with a satisfactory activity, e.g. for industrial use.

A further advantage is the possibility of carrying out protein/enzyme purification at high speed. The time saving compared with the processes of the prior art can be reckoned as equivalent to a factor of 2 to 5. Another advantage is that a relatively small volume is needed for elution.

As an alternative to the isolation of the proteins/enzymes, the enzyme adsorbed on the carrier material may be used directly as an immobilised enzyme after suitable crosslinking. (Cf. Ullmann's

Encyclopedia of Industrial Chemistry, 4th edition, vol. 10, pg. 542, Verlag Chemie 1975).

The proteins produced on an industrial scale, particularly the enzymes, are generally obtained from biomass. The literature gives sufficient information on the production of biomass from which the enzyme penicillinamidase (3.5.1.11) can be obtained (see for example H. J. Rehm & G. Reed in Biotechnology vol. 7a, VCH 1987; Ullmann's Encyclopedia of Industrial Chemistry, 5th Ed. vol. A9, pg. 371-382; VCH 1987). For working up, the biomass has to be homogenised, for example by the French press method using a French pressure cell press (SLM-Aminco) or using the Manton-Gaulin high pressure dispersion apparatus (cf. Ullmann's Encyclopedia of Industrial Chemistry, 4th edition, vol. 10, loc.cit., pp. 493-495). After homogenization, the enzyme solution is separated by centrifuging or filtering as already described above.

The present invention is illustrated by means of the non-limiting examples which follow:

EXAMPLES

Example 1

Preparation of phenyl butylamine substituted ® EUPERGIT C (PbA-EUPERGIT).

50 ml of 98% phenyl butylamine in 1325 ml of absolute ethanol are placed in a 4 litre three-necked round-bottomed flask and 250 g of dry EUPERGIT C are added with stirring (blade stirrer). This suspension is refluxed for 4 hrs. with gentle stirring. Then the material is cooled slightly and transferred to a G1 frit and washed with about 3 l of industrial ethanol and about 7 l of Millipore water in small batches (500 ml). After intensive suction filtering, the material is suspended once with 0.5 M sulphuric acid (then left to react) suction filtered again and recycled into the reaction flask with 750 ml of 0.5 M sulphuric acid. This suspension is refluxed for 30 minutes. Then the material is washed on the G1 frit with Millipore water to remove any acids (about 10 l). The material is sterilised by heating to 121°C for 30 minutes.

Example 2

Purification of penicillinamidase from E.coli by affinity chromatography.

Column material: phenyl butylamine-substituted ® EUPERGIT C (PbA EUPERGIT C) according to Example 3.

At a rate of 1 ml per minute (= 76.4 cm./hr. of linear flow) 15 ml of supernatant from E.coli homogenate (10.7 mg/ml of protein, 39 U/ml. of moist carrier activity) are applied to a column (volume: 8 ml; 1 cm. in diameter) in buffer A (0.05 M potassium phosphate

buffer, pH 7.5 plus 1 M NaCl), then washed with 30 ml of buffer A and the enzyme is eluted in 35 ml using buffer B (0.1 M sodium formate buffer pH 3.8). The yield of enzyme activity is 89%, the specific activity is increased from 2.0 U/mg in the supernatant homogenate to 10.2 U/mg in the eluate. In order to expose the enzyme to the acidic elution buffer for only a short time, 1 ml of potassium phosphate buffer (1 M, pH 7.5) is added to the fractions (5 ml in each case) of the eluate. The column is then regenerated with 40 ml of a solution of 0.006 Anson units of bacterial alkaline protease in 0.02 M glycine-NaOH buffer, pH 10, and washed until neutral with 60 ml of buffer A, before a new cycle is started.

Example 3

Immobilization of penicillinamidase on EUPERGIT C.

The penicillinamidase (PcA) purified over a PBA-Eupergit column was dialysed with water and evaporated down using a rotary evaporator. Since the PcA was very cloudy, it was centrifuged at 20,000 rpm for 15 minutes at 4°C. The supernatant was then clear.

Measurement of activity

The PcA is diluted 1:10 with 0.1 M potassium phosphate buffer (pH 7.5) (at about 180 U/ml). The enzymatic activity was determined against penicillin-G-potassium (crude) as substrate by alkalimetric titration at pH 7.8. 20 ml of a 2% substrate solution in 0.05 M potassium phosphate buffer (pH 7.8) were used and titrated at 37°C with 0.5 M NaOH.

Immobilization

510 U PcA were made up to a total volume of 4 ml with

1.5 M potassium phosphate buffer, pH 7.5, and then poured onto 1 g of Eupergit C. The mixture was left to stand at 23°C for 72 hrs. Then the mixture was washed three times on a frit with 0.1 M potassium phosphate buffer at pH 7.5.
Moist yield: 3.49 g
Activity: 91 U/g

Example 4

Measurement of activity of immobilized penicillin-amidase.

Principle

This is based on the titration of liberated phenyl acetic acid which is produced during the enzymatic hydrolysis of penicillin-G-potassium at pH 7.8 and 37°C.

Apparatus

Recording pH-stat. autotitration system, made by Radiometer-Copenhagen A/S or Metrohm SA (Switzerland)
Automatic Burette
pH-Meter
Titrating level
Servograph
Reaction vessel (100 ml in capacity) with a temperature-controlled outer jacket and stirring mechanism (not a magnetic stirrer which destroys the beads).
Thermostat.

Method

500 mg of EUPERGIT-PcA are washed several times with deionized water and then placed in a reaction vessel adjusted to a temperature of 37°C. 20 ml of substrate

solution heated to 37° are poured onto the enzyme carrier (substrate: 2% penicillin-G-potassium or -sodium (crude) are dissolved in 0.05 M potassium phosphate buffer, pH 7.5, and 0.05% of ethyl hydroxybenzoate are added to the solution). With constant stirring, 0.5 M NaOH is added through an automatic burette, controlled by a pH stat. to pH 7.8. At the same time the consumption of NaOH is recorded against the reaction time (first measurement). Incubation is stopped after 10 minutes, the enzyme carrier is placed in a glass frit (P2 or P3) and suction filtered with vacuum and washed three times with 2 ml of 0.1 M potassium phosphate buffer, pH 7.5. The washed beads are returned to the reaction vessel and the activity is determined (second measurement) as described above. The measurement of activity is repeated once more (third measurement) with the sample obtained in this way.

Calculation of the activity of the immobilised catalyst

The activity of the enzyme carrier is given in U/g of moist product. A Unit is defined as µMol of hydrolysed penicillin-G per minute (U = µMol/min.). 1 ml of 0.5 M NaOH corresponds to 500 µMol of hydrolysed penicillin-G. The linear path of the curve is used to determine activity (usually for the section between the 1st and 5th minutes). The activity of the immobilised catalyst is obtained from the average activity of the second and third incubations.

Example 5

Immobilization of the enzyme penicillinamidase from PbA-EUPERGIT by crosslinking.

10g of PbA EUPERGIT (moist weight) are washed three times in 10 volumes of 1 M phosphate buffer, pH 7.5, and

suction filtered over a vacuum on a glass frit. The corresponding moist material is placed in a glass beaker with 20 ml of an E.coli homogenate according to Example 6 with a penicillinamidase activity of 39 U/ml and the mixture is shaken for 60 minutes at 21°C. The product is then separated off by filtration (glass frit/vacuum), washed twice with 1.0 M potassium phosphate buffer (pH 7.5) and then suspended in 10 ml of 1.0 M potassium phosphate buffer. Then 0.250 ml of a 25% aqueous glutardaldehyde solution are added (stabilised over an ion exchange resin ® amberlite A 21). The suspension is shaken for 2 hrs., then washed three times with 0.05 M potassium phosphate buffer (pH 7.5). Moist yield 9.8 g. Measurement of activity gave a 62% activity yield and a product with 49 U/g of moist weight.

Example 6

Recovery of crude extract from Escherichia coli cells.

20 l of a fermenter culture solution was centrifuged at 4°C for 20 minutes (10,000 g, cryofuge, Heraeus). After the supernatant had been decantered, the moist cell mass was suspended in 900 ml of 0.05 M potassium phosphate buffer, pH 7.5, and centrifuged again. After the washing process, the moist cell mass (550 g) was suspended again in 1,200 ml of 0.05 M potassium phosphate buffer, pH 7.5. The cell suspension was frozen at - 80°C before the break-up of the cells. The E.coli cells have an activity of 58 U/g of cells in the presence of a 2% penicillin-G solution (20 ml, 37°C).

The break-up of the cells was carried out using the French press method (1000 bar pressure difference, French pressure cell press, SLM-Aminco). To avoid losses of activity, the suspension was only thawed in

ice water shortly before the break up of the cells.

Aliquots of 40 ml of cell suspension were all homogenized four times in order to obtain the maximum activity of the homogenized material. After four successive cycles, the homogenate had an activity of 23 U/ml in the presence of a 2% penicillin-G solution (20 ml, 37°C). The homogenate was frozen directly after the break up of the cells at -80°C. In order to remove the cell debris the thawed homogenate was centrifuged at 4°C for 30 minutes (10,000 g, cryofuge Heraeus). The supernatant can be used directly for the subsequent chromatographic isolation of penicillin amidase (activity of supernatant 18.75 U/ml).

Claims:

1. A process for purifying proteins which are capable of binding to substrates which comprise aromatic groups, said process comprising fixing said proteins from an aqueous solution thereof by hydrophobic adsorption onto a specific carrier material having phenyl butylamine groups covalently bound thereto.

2. A process as claimed in claim 1 wherein said proteins are enzymes.

3. A process as claimed in claim 1, wherein said phenyl butylamine groups are covalently bound to said specific carrier material as the product of the reaction of a glycidyl group as the activated group of a carrier precursor T-Vs-A.

4. A process as claimed in any of the preceding claims, wherein the activated carrier precursor T-Vs-A is a crosslinked copolymer synthesized from the matrix monomers acrylamide, methacrylamide, the active binding monomers glycidyl acrylate, glycidyl methacrylate, and the crosslinking monomers N,N'-methylene-bis-acrylamide and N,N'-methylene-bis-methacrylamide.

5. A process as claimed in claim 3 or claim 4, wherein the portion of active binding monomers in the carrier precursor T-Vs-A is from 4 to 40% by weight.

6. A process as claimed in claim 4 or claim 5, wherein the proportion of crosslinking monomers in the carrier precursor T-Vs-A is 5 to 80% by weight.

7. A process as claimed in any of the preceding claims, wherein the ligand density of the fixed phenyl butylamine is 10 to 2,000 μmol per gramme of carrier

material.

8. A process as claimed in any of the preceding claims, wherein the protein is the enzyme penicillinamidase (E.C.3.5.1.11).

9. A process as claimed in any of the preceding claims, wherein the proteins fixed to said specific carrier material are eluted by means of a slightly acidic buffer solution and the eluate is readjusted to a pH value close to neutral.

10. A process as claimed in claim 9, wherein said specific carrier material is treated with a protease after elution of the proteins and is then washed.

11. A process as claimed in claim 10, wherein an alkaline protease in an alkaline buffer is used as the protease and subsequently the specific carrier material is washed with a buffer in the neutral range.

12. A process as claimed in claim 1 substantially as herein described with reference to the Examples.

13. Proteins when purified by a process as claimed in claim 1.

14. A process for permanently immobilising the enzyme penicillinamidase, wherein the enzyme fixed to the specific carrier material by hydrophobic adsorption according to claim 3 is crosslinked by means of chemical agents.

15. Process as claimed in claim 14, wherein glutardialdehyde is used as the chemical agent for crosslinking.

16. A process as claimed in claim 14 substantially as herein described with reference to the Examples.

17. A carrier material for fixing proteins, said carrier material having phenyl butylamine groups covalently bound thereto as the product of the reaction of a glycidyl group as the activated group of a carrier precursor T-Vs-A.

18. A carrier material as claimed in claim 17 wherein said proteins are enzymes.

19. A carrier material as claimed in claim 17 or claim 18 wherein the activated carrier precursor T-Vs-A is a crosslinked copolymer synthesized from the matrix monomers acrylamide, methacrylamide, the active binding monomers glycidyl acrylate, glycidyl methacrylate, and the crosslinking monomers N,N'-methylene-bis-acrylamide and N,N'-methylene-bis-methacrylamide.

20. A carrier material as claimed in any one of claims 17 to 19 wherein the portion of active binding monomers in the carrier precursor T-Vs-A is from 4 to 40% by weight.

21. A carrier material as claimed in claim 17 substantially as herein described and with reference to the Examples.